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The Impact of Stream Landscape on Genetic Structure and Dispersal Patterns in Stream Salamander (*Pachyhynobius shangchengensis*)

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Abstract Compared with other terrestrial environments, the stream environment generally presents a linear spatial structure and relatively simple environment. In a stream landscape, the dispersal direction of stream-type organisms usually presents a linear structure along the stream, which results in the limited dispersal and the genetic differentiation of stream-type organisms across different stream sections. The Shangcheng Stout Salamander (*Pachyhynobius shangchengensis*) is a narrowly distributed stream salamander in Dabie Mountains of East China. In the present study, we tested for the impact of stream landscape (i.e. waterfalls and underground river) on genetic structure and dispersal pattern in *P. shangchengensis* based on 12 nuclear microsatellite loci from 195 individuals in 3 populations (A, B and C) from three closely connected sections within one stream. Genetic diversity results suggested that Population B contains relatively high genetic diversity for *P. shangchengensis* when compared to the other populations (A and C). Detectable genetic differentiation was found (F_{ST} = 0.008, P = 0.007) among three populations, which was also supported by the Structure, FCA analysis and relatedness estimates of each pair of individuals among populations. The assignment test suggested that *P. shangchengensis* has philopatric males and female-biased dispersal (mean female Alc = –0.031, SE = 0.225; mean male Alc = 0.026, SE = 0.198). Female-biased dispersal was also supported by analyses for each sex (i.e. Spatial autocorrelation, Genetic distance, Relatedness analysis). Our study indicated that small and isolated populations (A and C) had relatively low genetic diversity due to the limited population size. For stream salamanders, landscape features (i.e. waterfalls and underground river) can influence the ability of an individual to disperse through the landscape, and consequently influence the formation of strong genetic differentiation of *P. shangchengensis*.

Keywords Dabie Mountains, genetic structure, landscape features, *Pachyhynobius shangchengensis*, sex-biased dispersal

1. Introduction

Streams are fractal-like, occur with a hierarchical linear structure where smaller stream channels join to form larger ones in a dendritic pattern that resembles branches on a tree (Horton, 1945). It is because of the hierarchical linear structure of streams that environments and resources differ across the dendritic streams. The spatial heterogeneity of this environment and resources have led to discrete transitions in morphological, physiological, and behavioral traits (Wilbur, 1980; Moyle and Cech, 1988; Duellman and Trueb, 1994; Lowe *et al.*, 2004; Mullen *et al.*, 2010). It may have facilitated the formation of different dispersal patterns and genetic differentiation within different stream sections (Finn *et al.*, 2006; Muneepeerakul *et al.*, 2008; Grant *et al.*, 2009; Mullen *et al.*, 2010). Therefore, in a stream landscape, studies focusing on how factors (e.g. resource richness, stream

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landscape characteristics) have affected landscape-scale population structure and scaling of dispersal are critical for testing general models (Lowe *et al.*, 2006; Campbell Grant *et al.*, 2007; Mullen *et al.*, 2010).

Intraspecific interactions, competitive ability, habitat characteristics, and resource distribution and availability can influence dispersal patterns (Clobert *et al.*, 2001; Handley and Perrin, 2007). The dispersal patterns of species function as the key influence on genetic structure and help maintains genetic diversity (Bowler and Benton, 2005; Clobert *et al.*, 2001; Orsini *et al.*, 2013; Smith *et al.*, 2016; Olah *et al.*, 2017). When the evolutionary pressures between sexes differ, asymmetric dispersal of the sexes may occur, called sex-biased dispersal (Bonte *et al.*, 2011). Several theories have been generated to explain the differences in dispersal patterns between different sexes, such as local resource competition theory (Greenwood, 1980), local mating competition theory (Dobson, 1982) and avoidance of inbreeding theory (Pusey, 1987). In vertebrates, mammals usually show male-biased dispersal and birds typically show female-biased dispersal (Greenwood, 1980; Pusey, 1987; Handley and Perrin, 2007). As to the sex-biased dispersal reviewed in fish (Bekkevold *et al.*, 2004; Stiver *et al.*, 2007), amphibians (Palo *et al.*, 2004; Liebgold *et al.*, 2011; Wang *et al.*, 2012) and reptiles (Dubey *et al.*, 2008), the dispersal patterns and mating strategies within these taxa are diverse.

Unlike other landscape types, in stream landscapes, stream-type organisms are usually confined to rivers and streams, which leads to present a linear dispersal pattern along the waterways. As described in the above paragraph, previous studies have shown that some stream-dwelling organisms own different evolutionary, demographic and ecological processes across the dendritic streams (Finn *et al.*, 2006; Muneepeerakul *et al.*, 2008; Grant *et al.*, 2009; Mullen *et al.*, 2010). Therefore, to provide important evidence for the correlation between the stream landscape and the formation of different evolutionary, demographic and ecological processes in hierarchical linear structure, more work is needed that focuses on the dispersal patterns of stream-type organisms.

Population connectivity is critical to many ecological processes and conservation goals, and can be a key factor affecting the regional viability of populations (Curtis and Bevers, 2002; Hayden, 2006; Saastamoinen *et al.*, 2018). For amphibians, there are several types of landscape features, such as the presence of wetlands, changes in slope and cover type, that should influence the dispersal and population genetic structure (Spear *et al.*, 2005). Amphibians generally require wet areas to survive and reproduce (Duellman and Trueb, 1994), and therefore the presence of wetlands should be important for maintaining gene flow. Differences in slope among sites can account for the changes in dispersal or gene flow, as high levels of amphibian population subdivision exist among populations separated by mountains compared to low subdivision within basins in these populations (Funk *et al.*, 2005), ostensibly due to increased slope and reduced mobility. In fact, the effects of slopes and topography have been identified as the main predictors of amphibian dispersal or gene flow (Funk *et al.*, 2005; Olah *et al.*, 2017). Cover type may have a strong influence on landscape connectivity for amphibians. Intermittent landscape features directly affect population dispersal or gene flow (Spear *et al.*, 2005; Talbot *et al.*, 2017). Landscape genetics uses genetic data as the dependent variable to correlate genetic relationships with several independent variables representing natural, physical obstacles to address some of the most challenging ecological and evolutionary questions (e.g., source-sink dynamics, barriers and corridors, influence of landscape change) (Storfer *et al.*, 2007; Saastamoinen *et al.*, 2018). In a linear stream landscape, there are many landscape factors, such as waterfall, underground river, changes in slope and cover type, that can cause the stream discontinuity. For stream-type organisms, linear hierarchical networks with landscape barriers may prevent local adaptive populations from being submerged and promote genetic differentiation (Lowe *et al.*, 2006; Campbell Grant *et al.*, 2007; Campbell Grant *et al.*, 2010; Mullen *et al.*, 2010).

The Shangcheng Stout Salamander (*Pachyhynobibus shangchengensis*) is a narrowly distributed salamander endemic to streams above 500 meters in the Dabie Mountains, Eastern China (Fei *et al.*, 2010). It has deep genetic divergence among regional populations on a small spatial scale (Zhao *et al.*, 2013; Pan *et al.*, 2014, 2019). For narrowly distributed species with deep genetic divergence, such as *P. shangchengensis*, how it dispersal across those nature physical obstacles, such as waterfalls and underground rivers, in a linear river system, will help the understanding of how physical obstacles allow or restrict dispersal and create opportunities for genetic differentiation, then led to the deep genetic divergence. Therefore, in this study, we use an individual-based genetic approach with 12 microsatellite loci to assess how stream landscape features (e.g. waterfall, underground river) potentially influence the dispersal pattern and genetic structure of *P. shangchengensis* in Yaoluoping National Nature Reserve of Dabie Mountains.

2. Materials and Methods

2.1. Ethics statements The collection of samples was performed within a long-term investigation project on amphibians of Dabie Mountains. This investigation project and the sample collection were approved by Anhui Yaoluoping National Nature Reserve, Anhui Province, China. Additionally, the protocol was approved by the ethics committee of Anhui Normal University.

2.2. Study area and sampling Our study was conducted in the Yaoluoping National Nature Reserve (30°57'57" N, 116°4'5" E) in Southern Dabie Mountains, Yuexi county, Anhui Province in Eastern China (Figure 1). Dabie Mountains are located in the ecotone of subtropical evergreen broad-leaved forest and warm-temperate deciduous broad-leaved forest zone (Zheng *et al.*, 2012). *Pachyhynobius shangchengensis* is widely distributed throughout the streams and has a relatively large population in Yaoluoping National Nature Reserve, which will facilitate the population recovery after our sampling.

From August 26 to August 28, 2013, our study was carried out in three river sections (about 600 m; A, B and C) of the Yangtianwo valley area in the Yaoluoping National Nature Reserve (Figure 2). The streams have slight alkalinity (pH of 7.3), moderate midday temperatures (15.7 ℃), river flow velocity (0.028–0.669 m/s). The B and C sections are part of the main river, which are isolated from each other by a waterfall (15 m height); section A is a tributary of the main river. The landscape between sections A and C is an underground river, which is mostly covered by vegetation section (10 m) without a pool of water. The landscape details for sampling along the river are shown in Figure 2.

All adult individuals were captured by dip netting with relative positions recorded with a 500-meter Engineering ruler. Muscle or liver tissue was sampled and preserved in 100% ethanol for DNA extraction later. Specimens were fixed in 10% formalin and subsequently transferred to 75% ethanol for storage. All the specimens were deposited

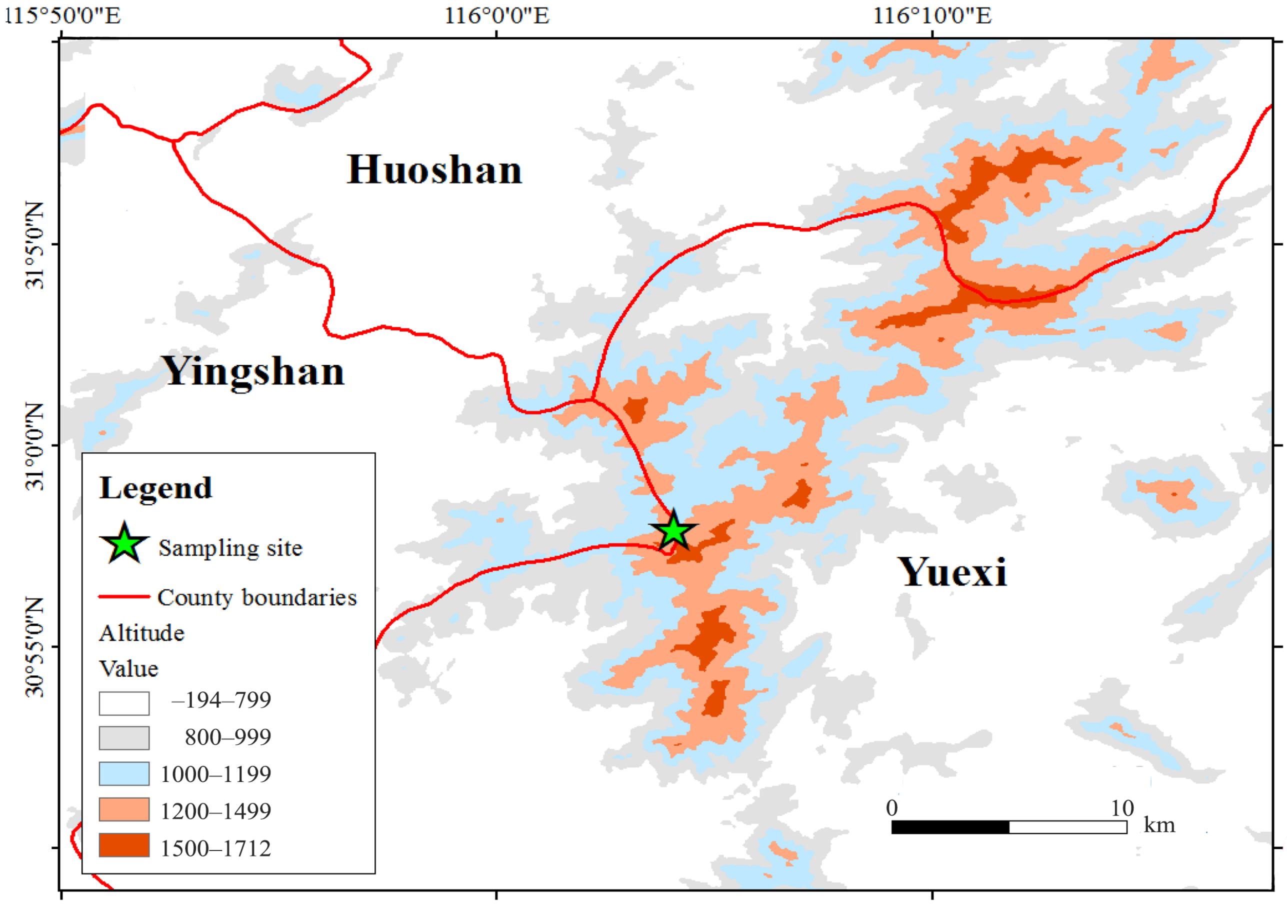

Figure 1 Sampling area for the populations of *Pachyhynobius shangchengensis*. The red line represents the county line. Values with different colors represent altitudes. The green star corresponds to sample areas. Huoshan County and Yuexi County are located in the Anhui Province. Yingshan County is located in the Hubei Province.

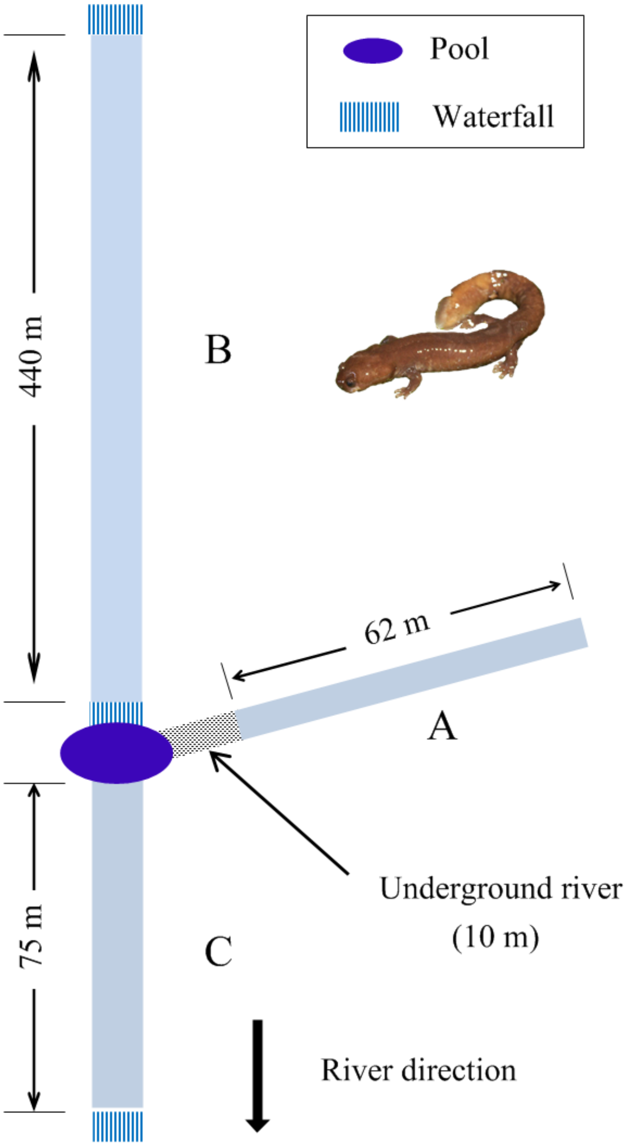

Figure 2 The schematic diagram of the sampled river in Yangtianwo of Yaoluoping area, Yuexi Country, China. The width of the sampled river is no more than 2 meters. The B and C sections are part of the main river, which are isolated from each other by a waterfall (15 m height); section A is the tributary of main river. The landscape between sections A and C is the underground river (10 m).

at the Zoological Specimen of Museum Anhui University (AHU), Hefei, Anhui, China. Sex was determined through sexually dimorphic features between sexes, such as the head length and width (Clemen and Greven, 2009) and the presence of reproductive system (Bulakhova and Berman, 2013; Vadim *et al.*, 2015).

2.3. Lab work Total DNA was extracted from samples using a standard proteinase K/ phenol-chloroform protocol (Sambrook *et al.*, 1989). An EasyPure PCR Purification Kit (TransGene) was used to purify each DNA extraction. Twelve independent polymorphic microsatellite loci for *P. shangchengensis* were selected from previous studies (Table 1) (Wang *et al.*, 2010). PCR was performed in 20 μL reactions containing 20–50 ng of template DNA, 10 μL 2 × Easy*Taq* PCR SuperMix polymerase (TransGen Biotech, containing 1.25U Ex *Taq*, 0.4 mmol/L dNTP, 4 mmol/L Mg^{2+}) and 0.2 μmol/L of each primer. Thermal cycling was performed with the following profile: 5 min denaturing step at 95 °C; followed by 35 cycles of 30s at 95 °C, 1 min at optimal annealing temperature (Wang *et al.*, 2010) and 1 min at 72 °C; and a final extension at 72 °C for 10 min. All PCR products were visualized on an ABI 3730 sequencer (PE Applied Biosystems) with GS500 marker. Allele peaks were scored using GeneMarker 1.85 (SoftGenetics LLC) (Holland and Parson, 2011).

2.4. Basic genetic information Number of alleles (*Na*), effective number of alleles (*Ne*), observed heterozygosity (H_O), expected heterozygosity (H_E) and fixation index (*F*) were estimated in GenAlEx 6.5 (Peakall and Smouse, 2006). Cervus 3.0 was used to assess the polymorphism information content (PIC) and the frequency of null alleles (Kalinowski *et al.*, 2007).

2.5. Population genetic structure To determine whether there is genetic substructure among these three populations (A, B and C), we performed a genetic clustering analysis using STRUCTURE v.2.3 (Pritchard *et al.*, 2000). The lengths of Markov Chain Monte Carlo iterations and burn-in were set as 1,000,000 and 100,000, respectively. We tested the range of possible clusters (K) tested from 1 to 10, and performed 10 independent runs for each K. The other parameters were set as following: admixture model and correlated allele frequencies between populations.

Analysis of molecular variance (AMOVA) in GenAlEx was used to partition genetic variation within and among these sampling sites and to estimate overall and pairwise population genetic differentiation (F_{ST}) (Wright, 1965; Excoffier *et al.*, 1992; Peakall and Beattie, 1995). In order to assess if there was any bias in F_{ST} estimation given the uneven sample size in Population B, we performed an AMOVA on randomly subsampled sets of 20 samples from each population using the 'shuffle' function of GenAlEx. Tests for genetic differentiation were performed by 1000 random permutations. For probing the genetic structure, we conducted the factorial correspondence analysis (FCA) by the GENETIX 4.05 (Belkhir *et al.*, 1996–2004). The genetic differentiation among geographical samples was calculated by the standardized statistics F'_{ST} (Meirmans,

Table 1 Average genetic variation of twelve polymorphic microsatellite loci for *Pachyhynobibus shangchengensis*.

Locus	Size range	*Na*	*Ne*	H_O	H_E	*F*	F_{IS}	PIC	HWE
PSH29	160–193	15	7.49	0.84	0.87	0.03	0.07	0.85	
PSH74	167–271	21	13.19	0.86	0.92	0.07	0.10	0.92	
PSH130	229–273	13	7.41	0.68	0.87	0.21	0.14	0.85	*A
PSH236	147–207	16	5.56	0.77	0.82	0.06	0.09	0.80	
PSH133	199–291	18	4.21	0.49	0.76	0.36	0.30	0.73	*C
PSH255	97–161	17	10.56	0.85	0.91	0.06	0.001	0.89	
PSH422	126–204	19	11.29	0.81	0.91	0.12	0.19	0.91	
PSH463	168–228	15	10.86	0.73	0.91	0.20	0.13	0.90	*A
PSH493	156–212	13	8.40	0.81	0.88	0.08	0.02	0.86	
PSH146	184–270	27	13.02	0.90	0.92	0.02	0.03	0.92	
PSH118	161–177	10	4.41	0.48	0.77	0.38	0.46	0.73	*C
PSH131	116–138	6	1.58	0.29	0.37	0.20	0.23	0.33	*A
Mean		15.83	8.17	0.71	0.83	0.15	0.15	0.81	

Notes: Presented are locus code, fragment size ranges, number of alleles (*Na*), effective number of alleles (*Ne*), observed heterozygosity (H_O), expected heterozygosity (H_E) and fixation index (*F*). *The populations significant departure ($P < 0.01$) from *HWE*.

2006) and Jost's *Dest* (Meirmans and Hedrick, 2011) included in GenAlEx.

2.6. Sex-biased dispersl analyses Assignment tests implemented in GenAlEx (Favre *et al.*, 1997; Mossman and Waser, 1999) were used to investigate the pattern of sex-biased dispersal in *P. shangchengensis*. A log likelihood assignment test value is calculated for each individual and then the Assignment Index correction (AIC) score is calculated by subtracting the mean log likelihood of the population from an individual's log likelihood score. For each population AIc values will average zero, while individuals with negative AIc scores are considered to have a higher probability of being immigrants (Mossman and Waser, 1999). The significance of sex-biased dispersal was examined using Mann Whitney *U* tests according to the difference between sexes in the frequency distribution of AIc scores. A negative score indicates male-biased dispersal and a positive value is indicative of female-biased dispersal (Mossman and Waser, 1999). The females should be more related to proximate females than distant females when females are philopatric, and the opposite pattern should occur if males are philopatric.

To further evaluate the inbreeding pattern within or among populations, we calculated the relatedness estimate (r) (Wang, 2002) and the inbreeding coefficient (F_{IS}) for each pair of individuals (Ritland, 1996) by COANCESTRY 1.0.1.1 (Wang, 2011). In addition, we also estimated the pairwise relatedness values (Queller and Goodnight, 1989) and pairwise relationship (Kalinowski *et al.*, 2006) based on the allele frequencies for determining the distribution pattern of kinship among individuals within Population B by KINGROUP v.2 (Konovalov *et al.*, 2004). In addition, for further probing spatial genetic structure between males and females, we also calculated the codominant genotypic genetic distances by GenAlEx (Morin *et al.*, 1994; Taylor *et al.*, 1997) and the average relatedness (r) by COANCESTRY. If males are more related to proximate females, then inbreeding may occur (Lebigre *et al.*, 2010). For the genetic distance, we expect the philopatric sex to have significantly higher genetic distance values than the dispersing sex. Additionally, we investigated the proportions of genetic distance with population B for different distance classes among males and females, to account for the sex-biased difference in spatial genetic structure.

2.7. Spatial autocorrelation analysis Spatial genetic structure of *P. shangchengensis* was assessed using a multivariate spatial autocorrelation method, which strengthens the spatial signal by reducing stochastic noise resulting from the traditional allele-by-allele and locus-by-locus analyses (Smouse and Peakall, 1999). We calculated the squared genetic distance between two individuals following the method of Peakall and Beattie (Peakall and Beattie, 1995), and the sampling distance of each individual constituted the pairwise geographical distance. The autocorrelation coefficient (R) was calculated for a specified number of distance classes. The 95% confidence intervals for the estimate of R were generated by bootstrapping (1000 repeats). In this study, significant autocorrelation was declared only when r exceeded the permutation 95% confidence intervals around the null hypothesis of zero, and the bootstrap

95% confidence intervals around r did not exceed zero. Significant positive R implies that individuals within a particular distance class are genetically more similar than expected by random. Additionally, we conducted the Heterogeneity test to check the significant support of correlogram (Banks and Peakall, 2012). The spatial autocorrelation analysis was conducted including three parts (the entire sample with identified sexy males, and females) in GenAlEx.

3. Result

3.1. Basic population genetic information A total of 195 adults of *P. shangchengensis* were obtained from three sampling sections of river, including 20 individuals for Population A, 153 individuals for Population B, and 22 for Population C (Figure 2 and Table 2). According to sex characteristics, the number of identified individuals is 187, with 101 male individuals and 86 female individuals (A: 10 male, 10 female; B: 79 male, 68 female; C: 12 male, 8 female).

In this study, small percentage of the microsatellite loci significantly deviated from HWE (Population A: PSH130, PSH463, PSH131; Population C: PSH133, PSH118) (Table 1). The frequencies of null alleles varied from 0.0094 to 0.26 and no loci showed evidence of null alleles. All loci showed wide variation in allele size range (Table 1). The numbers of alleles per locus (*Na*) ranged from 6 to 27 with the average of 15.83 (Table 1), indicating relatively high allelic diversity. The observed and expected heterozygosities (H_O, H_E) were 0.29–0.90 (average 0.71) and 0.37–0.92 (average 0.83), respectively (Table 1). Moreover, the PIC values ranged from 0.33 to 0.92 (average 0.81) (Table 1), indicating that these markers are highly informative. In total, 12 microsatellite loci showed polymorphism (Table 1). Among three populations, the number alleles across loci was higher in population B (15.50) followed by population C (11.33) and population A (10.17) (Table 2). Additionally, the higher values of observed and expected heterozygosities were found in population B (H_O: 0.71; H_E: 0.83) and population A (H_O: 0.71; H_E: 0.79) followed by population C (H_O: 0.68; H_E: 0.78). The replicated genetic diversity analysis for relatively equal sample sizes across the three populations (pop A: 20, pop B: 20, pop C: 22) based on 18 replicates showed very similar results, indicating that Population B had the higher genetic diversity, no matter *Na* or and H_O and H_E (Table S1).

Table 2 Genetic diversity from three section of river (A, B and C population) in *P. shangchengensis* in China.

	n	*Na*	H_O ± SE	H_E ± SE	F_{IS} ± SE
A	32	10.17	0.71 ± 0.23	0.79 ± 0.14	0.128
B	156	15.50	0.71 ± 0.19	0.83 ± 0.16	0.145
C	22	9.67	0.68 ± 0.27	0.78 ± 0.15	0.151

Notes: Sampling size (*n*), Number of alleles (*Na*), Observed Heterozygosity (H_O), Expected Heterozygosity (H_E), inbreeding coefficient (F_{IS}).

3.2. Population genetic structure The AMOVA analysis revealed a low but significant level of genetic differentiation among the three populations (F_{ST} = 0.009, P = 0.001). Among the three populations, Population A was significantly statistical different from the other two populations (vs. population B F_{ST} = 0.010, P = 0.001; vs. population C F_{ST} = 0.014, P = 0.002). Moreover, Populations B and C were also significantly statistical different (F_{ST} = 0.008, P = 0.003). The replicated AMOVA analysis at relatively equal sample sizes across the three populations (pop A: 20, pop B: 20, pop C: 22) based on 18 replicates showed similar results, indicating little or no bias in estimation due to sample size variation (Table S2). The STRUCTURE results showed the significant genetic differentiation between population C and the other two populations (A and B) due to the proportion of genetic clusters and maximum ΔK (Figure 3 and Figure S1). On the other hand, when K=8, the genetic clustering composition in population A also has a certain degree of differentiation with the genetic clustering in population B, suggesting that there is also a significant genetic difference between these two populations (Figure 3). FCA also showed that the individuals from three populations experienced significant separation trends (Figure 4), indicated substantial genetic differentiation among these three populations. In addition, this genetic differentiation pattern also got the support from the results of genetic distance (Pair-wise Jost's *Dest* and F'_{st}) between populations (Table 3).

The relatedness estimates (r) and inbreeding coefficient (F_{IS}) exhibit a linear relationship (Figure 5). The inbreeding coefficient values within populations were larger than that those between populations, suggesting close relatedness within populations (Figure 5). According to relatedness estimates (r) and the inbreeding coefficient (F_{IS}) within the population, it seems that small populations will have larger inbreeding pressure. As for the three populations, Population B has a relatively small inbreeding coefficient, which indicates that individuals within the Population B might experience relatively longer dispersal distance (Table 4, Figure 5). In addition, the relatedness estimates (r) and the inbreeding coefficient (F_{IS}) between Population

Table 3 Pair-wise Jost's Dest (upper diagonal) and *F*'st (below diagonal) among three geographic populations of *P. shangchengensis.*

	A	B	C
A		0.040 (0.004)**	0.047 (0.032)*
B	0.004 (0.004)**		0.031 (0.018)*
C	0.005 (0.032)*	0.003 (0.017)*	

Note: Significant tests are indicated with asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$); Inside the brackets is the P value.

A and B were smaller than the equivalents between B and C, consistent with the than bigger genetic distance (F_{ST}) and population differentiation between population A and B (Table 4, Figure 5).

3.3. Dispersal pattern analysis Due to the limited number of identified sexed individuals in Populations A (n = 14) and C (n = 12), all dispersal pattern analyses were conducted on Population B (n = 120). In our study, the Assignment Index correction (AIC) across all populations showed that female-biased dispersal (mean male AIC = 0.026, SE = 0.198; mean female AIC = -0.031, SE = 0.225) (Figure 6). In addition, the mean of pairwise relatedness values between male individuals (mean r = −0.0405, SD = 0.1115) was higher than that of female individuals (mean r =−0.0404, SD = 0.1131), they both deviated significantly from zero, which further supported the female-biased dispersal pattern. The mean codominant genotypic genetic distances between male individuals (22.701) was higher than that of female individuals (22.236), which also supported the female-biased dispersal pattern due to significantly higher genetic distance values of the philopatric sex compared with the dispersing sex.

Spatial genetic autocorrelation analysis showed that the first distance class (0–70 m), the R value among males (R = 0.014, P = 0.111; Figure 7A) was higher than that among females (R = −0.0145, P = 0.929; Figure 7B) and among all individuals in B population (R = 0, P = 0.468; Figure 7C), which suggested that the autocorrelation value of the first distance class was not significant, but the autocorrelation value indeed was much larger than that in Figure 7B. Additionally, R value of the four distance class among males also show similar pattern. On the other hand, the Heterogeneity Test show that the correlogram of R value among males (Omega = 32.143, P = 0.006) was significant supported, but the other two show different results (females: Omega = 17.590, P = 0.129; all individuals: Omega = 19.163, P = 0.095). Combined these clues, the spatial autocorrelation analysis also hint the female-biased dispersal.

Further examination of spatial distribution of codominant genotypic genetic distances with different genetic classes also showed that in the first distance class (0–50 m), the proportion of large genetic distance in female pairs (e.g. 3 or 4) was higher than male pairs and

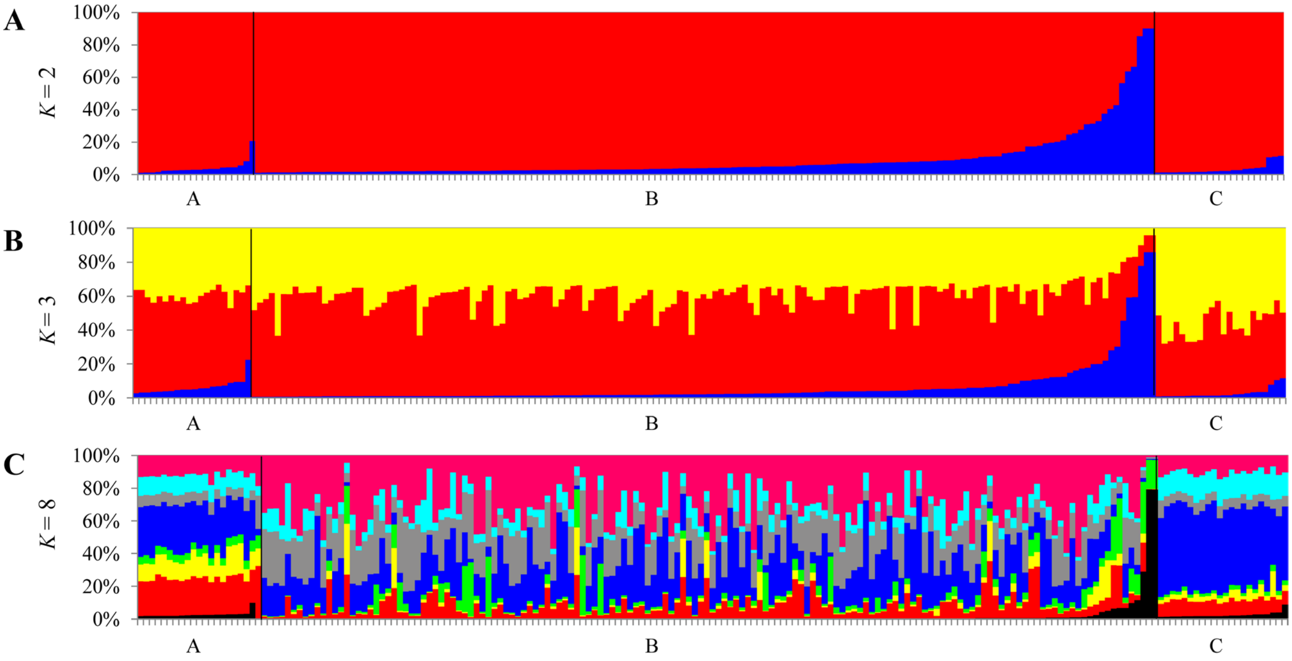

Figure 3 Bayesian population structure analysis of *P. shangchengensis*, on the basis of microsatellite genotyping data estimated by STRUCTURE. (A) output for K = 2, (B) output for K = 3, (C) output for K = 8. Different colors represent the proportion of each individual belonging to different putative clusters.

male–female pairs (Figure 8). Additionally, during those three distance classes (i.e., 301-350, 351–400, 401–451 m), the high genetic distances (e.g. 3 or 4) in male pairs occupy a extremely low proportion; however, in the female, it is the opposite situation. Theose results also hinted the female-biased dispersal in *P. shanchengensis* (Figure 8). In addition, according to the relationship percentage in population B, the percentage of female pairs with genetic relationship was smaller than that of male pairs, which also supported the female-biased dispersal (Table 5). In addition, following widely used criteria (0.25 ≤ *r* < 0.5, moderate inbreeding; *r* ≥ 0.5, high inbreeding) (Hansson *et al.*, 2007), we can infer that the most of relatedness values within population B were smaller than 0.25, which indicated that most of the individuals were out of inbreeding (Table S3).

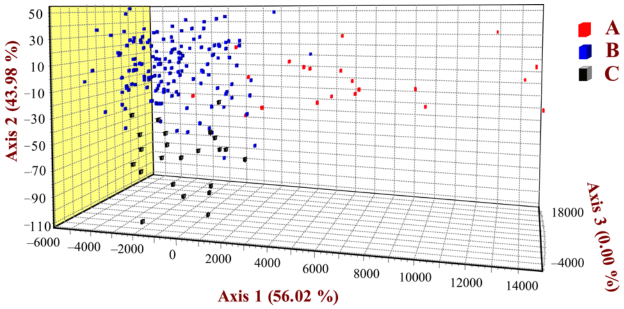

Figure 4 The factorial correspondence analysis across populations of *P. shangchengensis*. Three populations are indicated by different colors: red, population A; blue, population B; black, population C.

4. Discussion

4.1. Genetic diversity in isolated populations In conservation biology, population isolation often leads to a series of negative consequences, such as losing genetic diversity, reducing individual fitness, and increasing the risk of inbreeding, which are closely related to the species' viability and extinction risk (Ellstrand and Elam, 1993; Avise and Hamrick, 1996; Fang *et al.*, 2002; Keller and Waller, 2002; Burns *et al.*, 2004; Reed, 2005; Collier *et al.*, 2010; Hu *et al.*, 2010). In this study, Populations A, B and C are isolated from each other by a waterfall and underground river, Population A and C is smaller than Population B. Overall, Population B showed higher genetic diversity and a lower inbreeding coefficient than Populations A and C (Tables 2, 4 and Table S1). It appears that in *P. shangchengensis*, smaller populations harbor lower genetic diversity and suffer higher inbreeding risk as one would predict. Studies have shown that negative consequences, such as low genetic diversity and inbreeding, are caused by increasing difficulty for species to access resources, such as food, mates, home domain size (Fahrig, 2003; Balkenhol *et al.*, 2015). In this study, the landscape barriers (the waterfall and underground river) in the stream habitat produced discontinuous sections and have led to the heterogeneity in the distribution of resources (e.g. food, mate, home domain).

4.2. Landscape barriers and spatial genetic structure Due to limited dispersal or restricted gene flow, multiple factors may influence spatial genetic structure, including geographic barriers, risk associated with dispersal, environmental heterogeneity, population density, etc. (Koenig *et al.*, 1992; Bowler and Benton, 2005;

Table 4 The Statistical parameters of relatedness estimates (*r*) and the inbreeding coefficient (F_{IS}) of each pair of individuals within and between populations in *P. shangchengensis*.

	A–A		B–B		C–C		A–B		A–C		B–C	
	r	F_{IS}	*r*	F_{IS}	*r*	F_{IS}	*r*	F_{IS}	*r*	F_{IS}	*r*	F_{IS}
Average	0.0017	0.0221	0.0341	0.0094	−0.0483	−0.0326	0.0426	0.0116	−0.0680	−0.0345	0.1772	0.0752
SD	0.1346	0.0307	0.1757	0.1041	0.2454	0.1527	0.0525	0.0165	0.0659	0.0858	0.0523	0.0107
Median	−0.0029	−0.0067	0.0341	0.0094	−0.0483	−0.0326	0.0426	0.0116	−0.0680	−0.0345	0.1772	0.0752
95% HPD Lower	−0.0197	0.0172	0.0309	0.0075	−0.0799	−0.0523	0.0407	0.0110	−0.0743	−0.0427	0.1754	0.0748
95% HPD Upper	0.0231	0.0270	0.0372	0.0113	−0.0166	−0.0129	0.0445	0.0121	−0.0617	−0.0262	0.1790	0.0756

Table 5 The estimated relationship results by the highest likelihood within population B. Note: U, Unrelated; HS, Half Sibs; FS, Full Sibs; PO, Parent / Offspring.

	Relationship					Proportion			
	U	FS	HS	PO	Total	U	FS	HS	PO
Male pairs	2807	9	265	0	3081	91.107	0. 292	8.601	0. 000
Female pairs	2083	5	190	0	2278	91.440	0. 219	8.341	0. 000
Male-Female pairs	4925	11	435	1	5372	91.679	0. 205	8.098	0. 019

Saastamoinen *et al.*, 2018). Streams possess a dendritic hierarchical network structure, where smaller stream channels join to form larger ones in a dendritic pattern (Horton, 1945). For many stream-dwelling organisms, the consistent network architecture may form natural landscape barriers and may lead to local divergence of populations (Lowe *et al.*, 2006; Campbell Grant *et al.*, 2007; Campbell Grant *et al.*, 2010) that ultimately face different evolutionary and ecological processes in different stream sections (Finn *et al.*, 2006; Muneepeerakul *et al.*, 2008; Grant *et al.*, 2009; Mullen *et al.*, 2010). For example, the Idaho giant salamander (*Dicamptodon aterrimus*) shows

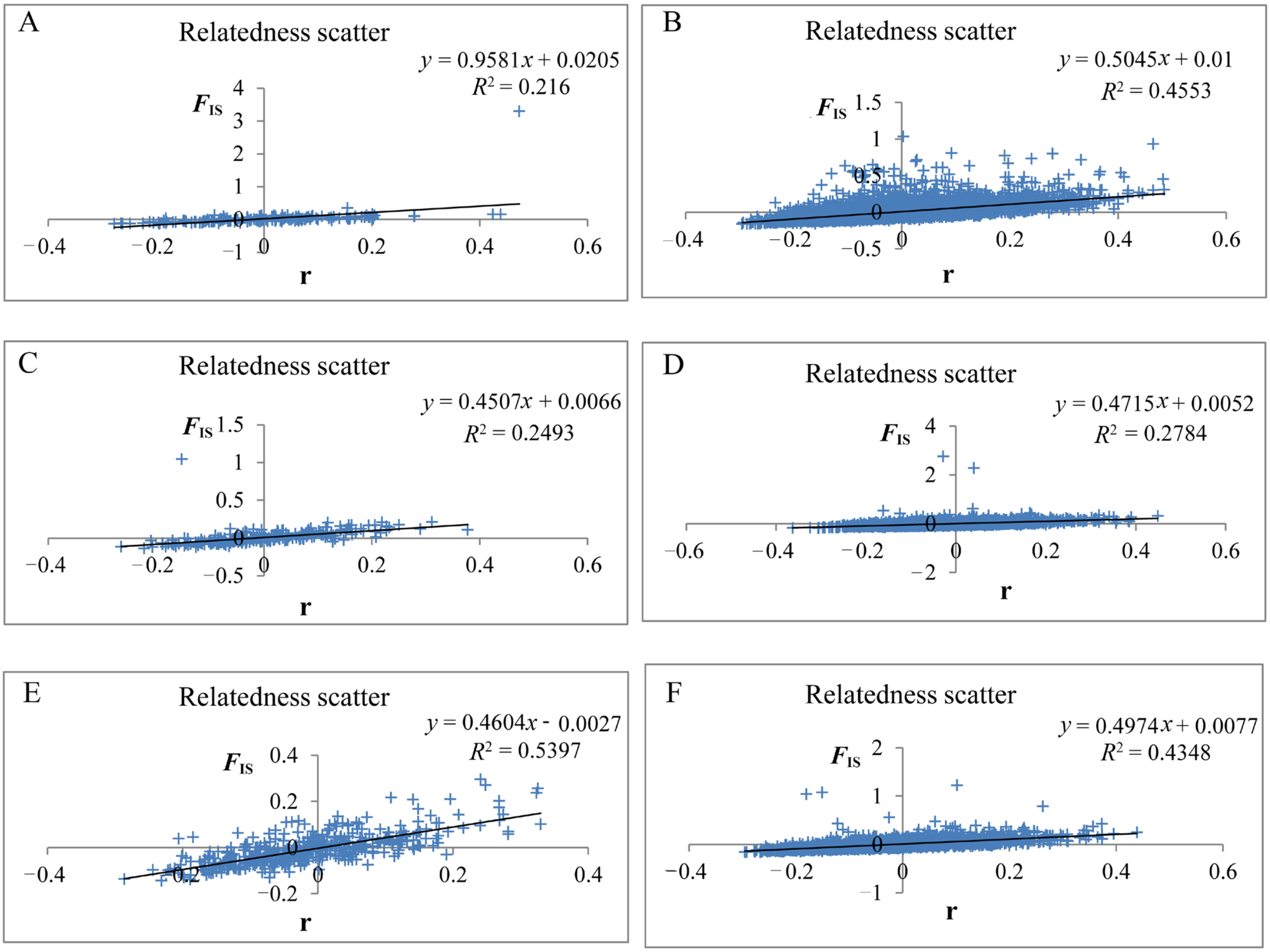

Figure 5 The relatedness scatter of relatedness estimates (r) and the inbreeding coefficient (F_{IS}) within or among population in *P. shangchengensis*. (A) within population A, (B) within population B, (C) within population C, (D) between population A and B, (E) between population A and C, and (F) between population B and C.

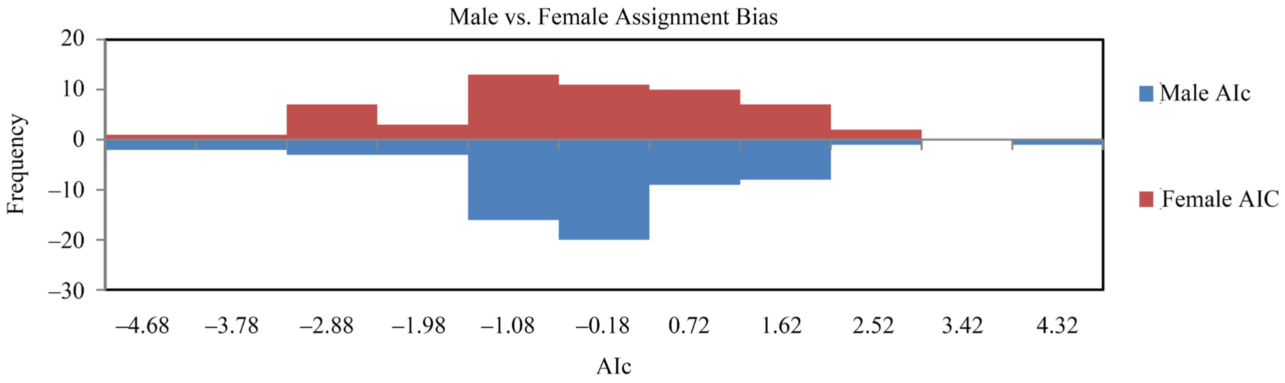

Figure 6 Frequency distribution of corrected assignment indices (AIC) of *P. shangchengensis.*

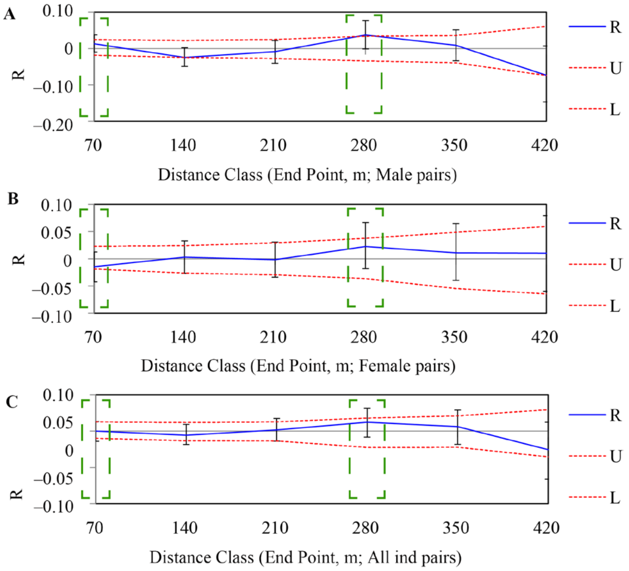

Figure 7 Spatial genetic autocorrelation analyses among (A) male pairs (n = 3081 pairs), (B) female pairs (n = 2,278 pairs), and (C) all individual pairs (n = 10,731 pairs) in Population B. The genetic autocorrelation coefficient (R) is a function of geographic distance, with the permuted 95% confidence intervals indicating random spatial genetic structure (dashed lines) and the bootstrapped 95% confidence error bars around r. U, upper 95% confidence interval; L, lower 95% confidence interval. The smallest distance class size was set as 70 m.

genetic differentiation among streams within catchments, consistent with a stream hierarchy model of population structure (Mullen *et al.*, 2010). On the scale of this stream network, the patterns of genetic differentiation were often driven by the limited migration across the network architecture (Argentina *et al.*, 2018; Jaisuk and Senanan, 2018).

At a smaller spatial scale, it is necessary to consider the influence of landscape factors on genetic pattern, especially landscape barriers that lead to discontinuous habitats, such as waterfalls and underground rivers in streams. Actually, the contiguous stream population that we assumed was composed of many smaller populations of different sizes (Jaisuk and Senanan, 2018). Whether landscape barriers substantially affect genetic structure needs further study. In this study, our data suggested that even at a smaller spatial scale, the main stream landscape barriers (waterfall and underground river) can still produce a clear signal of population genetic differentiation in *P. shangchengensis* (Table 3, Figures 3 and 4). Populations A, B and C, are isolated by the waterfall and underground river. Population connectivity is critical to many ecological processes and conservation goals (Curtis and Bevers, 2002; Hayden, 2006; Saastamoinen *et al.*, 2018). The key factor to population connectivity is dispersal, which greatly affected population genetic structure, population dynamics, population persistence and adaptability (Clobert *et al.*, 2001; Bowler and Benton, 2005; Orsini *et al.*, 2013; Smith *et al.*, 2016; Olah *et al.*, 2017). Generally, limited by the characteristics of the linear habitat, the stream-type organisms are usually sensitive to the landscape barrier that effectively forms discontinuous stream habitat (Lowe *et al.*, 2006; Campbell Grant *et al.*, 2007; Campbell Grant *et al.*, 2010). In this study, the genetic differentiation among Populations A, B, C indicate that waterfalls and underground rivers act as effective natural landscape barriers for *P. shangchengensis*. As a narrowly distributed species, our results suggest that the distribution of *P. shangchengensis* was affected by landscape factors, which may facilitate to understand the population divergence and evolutionary history of it.

4.3. Female-biased dispersal Sex-biased dispersal may occur when the evolutionary pressures between sexes are different (Bonte *et al.*, 2011). In recent years, sex-biased dispersal studies have been carried out in various vertebrate animal groups, such as fish (Bekkevold *et al.*, 2004; Stiver *et al.*, 2007), amphibians (Palo *et al.*, 2004;

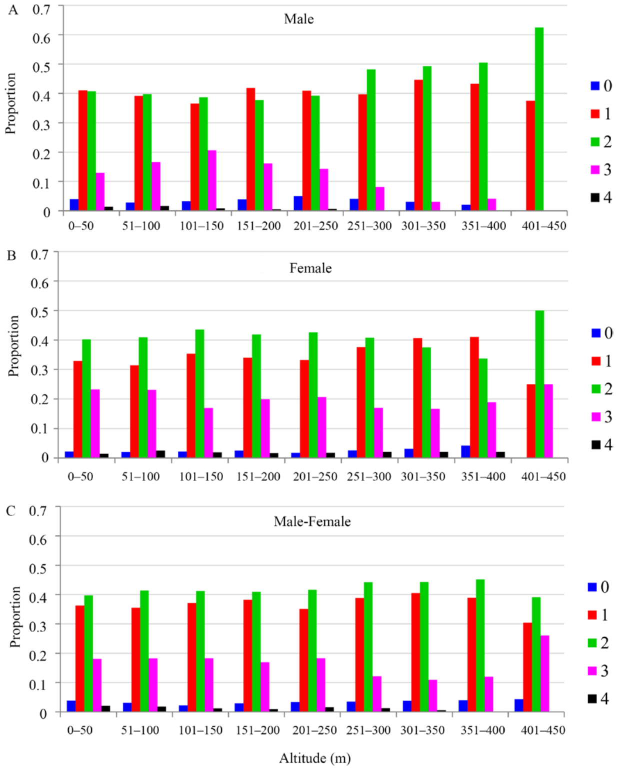

Figure 8 Spatial distribution of codominant genotypic genetic distances among (A) male pairs (n = 3081 pairs), (B) female pairs (n = 2278 pairs), and (C) male-female pairs (n = 5372 pairs) in Population B. The distance classes was set as 50 m. The genetic distance between individuals (0, 1, 2, 3 and 4) was calculated based on the method from Smouse and Peakall, (1999).

Liebgold *et al.*, 2011; Wang *et al.*, 2012), reptiles (Dubey *et al.*, 2008), birds (Paris *et al.*, 2016) and mammals (Höner *et al.*, 2008). Among those groups, mammals usually show male biased dispersal and birds typically show female-biased dispersal (Greenwood, 1980; Pusey, 1987; Handley and Perrin, 2007). However, the dispersal patterns and mating strategies were diverse in amphibians and reptiles. For example, the direct-developing salamander *Plethodon cinereus* showed females as philopatric and male-biased dispersal due to the local mating competition (Liebgold *et al.*, 2011); in the socially monogamous lizards of the genus *Egernia*, similar dispersal patterns were found (Gardner *et al.*, 2001; Stow *et al.*, 2001; Chapple and Keogh, 2005); In contrast, the dispersal pattern of the common frog (*Rana temporaria*) is female biased (Palo *et al.*, 2004). Female-biased dispersal has also been observed in the Columbia spotted frog (*R. luteiventris*) (Pilliod *et al.*, 2002) and the bullfrog (*R. catesbeiana*) (Austin *et al.*, 2003).

In this study, based on multiple analytical approaches (Figures 6, 7 and 8), including the Assignment Index correction (AIc), genetic distance, pairwise relatedness values of different gender (R) and spatial autocorrelation analysis, female-biased dispersal and male philopatry of *P. shangchengensis* was concluded. In nature, many animal groups show female-biased dispersal, such as fish (Taylor *et al.*, 2003), amphibians (Pilliod *et al.*, 2002; Austin *et al.*, 2003; Palo *et al.*, 2004), birds (Greenwood, 1980; Paris *et al.*, 2016; Pusey, 1987) and mammals (Hammond *et al.*, 2006; Nagy *et al.*, 2013; Tucker *et al.*, 2017). In anurans and birds, the general trend for female-biased dispersal had been proposed (Greenwood, 1980; Pusey, 1987; Pilliod *et al.*, 2002; Austin *et al.*, 2003; Palo *et al.*, 2004; Wang *et al.*, 2012; Paris *et al.*, 2016). Unlike the amphibian species above mentioned, *P. shangchengensis* is a stream=salamander. In this study, we found differences in dispersal distances between the sexes, indicating female-biased dispersal and male philopatry of *P. shangchengensis*. Our results enrich the basic dispersal pattern of amphibian research, especially for the aquatic stream salamanders. Future studies should focus on whether female-biased dispersal acts as the general trend for aquatic Caudata. Unlike the female-biased dispersal of anurans (Austin *et al.*, 2003; Lampert *et al.*, 2003; Palo *et al.*, 2004; Wang *et al.*, 2012), it seems that there are no consistent sex-biased dispersal patterns in Caudata, although previous studies reported the male-biased dispersal pattern of two terrestrial salamanders (alpine salamander, *Salamandra atra*; red-backed salamander, *P. cinereus*) (Liebgold *et al.*, 2011; Helfer *et al.*, 2012). For Caudata, different habitat types (e.g. terrestrial-type, stream-type) might have impacts on its dispersal patterns. Combined with the female-biased dispersal of *P. shangchengensis*, we think that there are multiple dispersal patterns (male-biased dispersal, female biased dispersal) corresponding to different habitat types. Therefore, for verifying the hypothesis, further research on dispersal patterns associated with habitat type may be needed. Additionally, the potential forces that facilitate or limit dispersal should also be studied to refine the understanding of the evolution of sex-biased dispersal.

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Appendix

Table S1 Genetic diversity across loci of population after shuffle in *P. shangchengensis* from three section of river (A, B and C population) in China.

	Pop		*Na*	*Ho*	H_{E}	F_{IS}
shuffle 1	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.42	0.73	0.81	0.1
		SE	1.05	0.06	0.05	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 2	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.92	0.75	0.81	0.09
		SE	0.96	0.05	0.04	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 3	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.92	0.71	0.82	0.15
		SE	1.18	0.05	0.04	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 4	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.83	0.7	0.79	0.11
		SE	1.2	0.06	0.06	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 5	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.75	0.73	0.8	0.11
		SE	1.08	0.06	0.04	0.05
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 6	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	11.42	0.73	0.82	0.09
		SE	1.15	0.04	0.05	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 7	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.75	0.73	0.8	0.08
		SE	1.16	0.06	0.05	0.05
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 8	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.33	0.72	0.8	0.11
		SE	1.09	0.05	0.04	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 9	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.58	0.73	0.81	0.09
		SE	1.19	0.05	0.05	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 10	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.67	0.7	0.79	0.12
		SE	1.07	0.06	0.05	0.05
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 11	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.42	0.68	0.81	0.17
		SE	0.98	0.07	0.04	0.06
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 12	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	11	0.73	0.82	0.11
		SE	0.93	0.05	0.04	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 13	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.42	0.7	0.8	0.11
		SE	1.13	0.07	0.05	0.07
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 14	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	11.08	0.75	0.82	0.09
		SE	1.1	0.06	0.04	0.05
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 15	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.75	0.74	0.82	0.1
		SE	1.29	0.06	0.05	0.04
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 16	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	11	0.71	0.8	0.12
		SE	1	0.06	0.05	0.05
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 17	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.58	0.71	0.81	0.12
		SE	0.96	0.05	0.05	0.03
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07
shuffle 18	A	Mean	10.17	0.71	0.79	0.12
		SE	1.04	0.07	0.04	0.06
	B	Mean	10.58	0.67	0.8	0.15
		SE	1	0.05	0.04	0.05
	C	Mean	9.67	0.68	0.78	0.16
		SE	1.01	0.08	0.04	0.07

Table S2 Results of F_{ST} after shuffle calculated from hierarchical AMOVA based on microsatellites of *P. shangchengensis.*

shuffle 1		A	B	C
	A	0.000	0.104	0.003
	B	0.006	0.000	0.095
	C	**0.015**	0.004	0.000
shuffle 2		A	B	C
	A	0.000	0.067	0.002
	B	0.006	0.000	0.009
	C	**0.015**	**0.011**	0.000
shuffle 3		A	B	C
	A	0.000	0.067	0.002
	B	0.006	0.000	0.006
	C	**0.015**	**0.012**	0.000
shuffle 4		A	B	C
	A	0.000	0.006	0.002
	B	**0.012**	0.000	0.003
	C	**0.015**	**0.015**	0.000
shuffle 5		A	B	C
	A	0.000	0.009	0.002
	B	**0.013**	0.000	0.001
	C	**0.015**	**0.018**	0.000
shuffle 6		A	B	C
	A	0.000	0.101	0.002
	B	0.006	0.000	0.013
	C	**0.015**	0.010	0.000
shuffle 7		A	B	C
	A	0.000	0.101	0.001
	B	0.006	0.000	0.137
	C	**0.015**	0.004	0.000
shuffle 8		A	B	C
	A	0.000	0.032	0.001
	B	0.008	0.000	0.002
	C	**0.015**	**0.014**	0.000
shuffle 9		A	B	C
	A	0.000	0.205	0.002
	B	0.003	0.000	0.076
	C	**0.015**	0.006	0.000

shuffle 10		A	B	C
	A	0.000	0.001	0.002
	B	**0.019**	0.000	0.001
	C	**0.015**	**0.020**	0.000
shuffle 11		A	B	C
	A	0.000	0.035	0.001
	B	0.008	0.000	0.066
	C	**0.015**	0.007	0.000
shuffle 12		A	B	C
	A	0.000	0.006	0.001
	B	**0.013**	0.000	0.066
	C	**0.015**	0.006	0.000
shuffle 13		A	B	C
	A	0.000	0.007	0.001
	B	**0.013**	0.000	0.041
	C	**0.015**	0.007	0.000
shuffle 14		A	B	C
	A	0.000	0.014	0.001
	B	0.009	0.000	0.005
	C	**0.015**	**0.010**	0.000
shuffle 15		A	B	C
	A	0.000	0.017	0.002
	B	0.010	0.000	0.037
	C	**0.015**	0.007	0.000
shuffle 16		A	B	C
	A	0.000	0.028	0.001
	B	0.008	0.000	0.017
	C	**0.015**	0.009	0.000
shuffle 17		A	B	C
	A	0.000	0.005	0.001
	B	**0.012**	0.000	0.001
	C	**0.015**	**0.021**	0.000
shuffle 18		A	B	C
	A	0.000	0.062	0.003
	B	0.007	0.000	0.003
	C	**0.015**	**0.014**	0.000

Table S3 The estimated relatedness among all individuals in population B.

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	F29	F30	F31
F1	1																														
F2	0.09	1																													
F3	0.04	0.09	1																												
F4	0	0	0.06	1																											
F5	0	0.29	0	0	1																										
F6	0	0.33	0.04	0	0.07	1																									
F7	0	0	0	0.04	0	0.25	1																								
F8	0	0	0	0	0	0.04	0.18	1																							
F9	0.12	0	0	0	0	0	0.08	0.09	1																						
F10	0.1	0.13	0.01	0	0.17	0	0	0	0	1																					
F11	0.03	0	0	0	0	0.15	0.03	0.07	0.08	0.05	1																				
F12	0.02	0	0	0.07	0	0	0	0	0.09	0	0.07	1																			
F13	0	0	0	0.2	0.04	0.04	0	0	0	0.02	0	0	1																		
F14	0.04	0	0.05	0	0	0.15	0.06	0	0	0.26	0.17	0	0	1																	
F15	0	0	0.02	0	0	0	0	0.16	0.18	0	0	0.16	0	0	1																
F16	0.1	0	0	0	0	0	0	0.03	0.04	0	0	0.14	0	0	0.14	1															
F17	0	0	0.04	0	0.04	0	0	0	0	0.22	0.01	0.04	0	0	0	0.22	1														
F18	0	0	0	0	0	0.08	0	0	0	0	0	0	0	0	0.05	0.09	0	1													
F19	0	0	0	0	0	0	0.17	0	0	0	0.13	0	0	0.01	0	0	0	0	1												
F20	0.04	0	0	0.04	0.01	0.12	0.06	0.12	0.06	0.08	0.07	0	0.09	0.08	0	0	0.02	0	0.02	1											
F21	0	0	0.09	0	0.01	0.08	0	0	0.01	0	0	0.17	0	0.01	0	0	0	0.11	0.23	0.01	1										
F22	0	0	0.02	0.27	0.26	0.01	0.01	0	0.03	0.05	0	0.01	0.1	0.09	0	0	0	0.07	0.01	0.04	0.03	1									
F23	0.02	0.12	0	0	0	0.06	0.08	0	0	0	0	0	0.22	0	0	0	0	0	0.08	0	0.04	0	1								
F24	0.11	0.33	0.07	0	0.09	0	0	0	0.23	0.01	0.07	0	0	0	0	0	0.01	0	0.09	0.01	0.06	0	0	1							
F25	0	0.06	0	0	0.05	0.01	0.01	0.07	0	0.19	0.09	0.17	0.04	0.08	0	0	0	0.08	0.01	0	0	0.01	0.01	0	1						
F26	0	0	0	0.06	0	0	0	0	0.01	0.14	0.04	0.08	0.16	0.12	0	0	0.05	0	0	0.08	0	0	0.13	0	0.29	1					
F27	0	0	0	0.05	0.03	0.18	0	0.07	0.05	0	0.1	0.05	0.13	0.03	0.14	0.01	0	0	0.08	0	0	0	0	0	0.07	0	1				
F28	0	0.04	0	0.07	0.07	0	0	0	0.06	0	0.08	0	0	0	0	0	0	0	0.02	0.06	0	0	0.07	0.08	0	0.06	0	1			
F29	0.04	0.05	0	0	0.09	0	0	0	0	0.2	0	0.05	0	0	0.02	0	0.05	0	0	0.07	0.08	0.01	0	0.06	0	0	0	0	1		
F30	0	0	0	0	0	0.07	0	0.1	0.06	0	0.25	0.05	0.03	0	0	0	0.15	0	0	0.04	0	0	0	0.02	0	0	0	0	0	1	
F31	0.1	0.11	0.11	0	0	0	0	0.07	0.22	0.07	0.09	0	0	0.05	0.18	0	0.01	0	0	0	0	0	0	0.29	0.19	0.07	0.07	0.14	0	0.13	1
F32	0	0.11	0.01	0.07	0.14	0.19	0.18	0.15	0.06	0	0.06	0	0	0	0.15	0.1	0	0	0.1	0	0.01	0.15	0.01	0.01	0.01	0	0.06	0.15	0	0.01	0
F33	0.03	0	0	0	0.04	0.06	0	0	0.04	0	0.06	0	0.04	0	0	0.11	0	0	0.08	0.07	0.02	0	0	0.06	0	0	0.05	0.05	0.16	0.09	0
F34	0	0	0	0.12	0	0.02	0.14	0	0.13	0	0	0.03	0.04	0	0	0	0	0	0.13	0	0.02	0.08	0	0	0	0	0	0	0.04	0.14	0.07
F35	0	0	0	0	0.05	0	0	0.06	0.03	0.02	0	0	0	0.07	0.05	0	0.18	0	0	0.1	0.06	0.09	0	0	0	0	0	0	0	0	0
F36	0	0.12	0	0	0.14	0.04	0	0	0	0.08	0	0.14	0.04	0	0.09	0.21	0.18	0.17	0	0	0	0	0	0	0.25	0.21	0.03	0	0.03	0	0
F37	0	0.13	0	0	0.11	0.01	0	0	0.05	0.08	0.05	0.15	0.13	0	0	0	0	0	0	0.09	0	0.01	0.06	0	0.29	0.42	0	0	0	0.04	0.08
F38	0	0	0	0.03	0	0.03	0	0	0.11	0	0.02	0	0.01	0.16	0	0	0.06	0.02	0.12	0.11	0.27	0.03	0	0.11	0	0	0	0.07	0	0.06	0.17
F39	0	0	0.01	0	0.08	0	0	0	0.06	0.16	0.09	0	0.13	0.23	0	0	0.07	0.11	0.08	0	0.19	0	0.16	0	0.16	0.29	0.1	0.06	0.1	0	0
F40	0	0	0.15	0	0	0	0	0.11	0	0	0.32	0.04	0	0.21	0.07	0.05	0.04	0	0.2	0	0.07	0	0.09	0.09	0.09	0.01	0.12	0.01	0	0.16	0.16
F41	0.11	0	0	0	0.13	0.04	0	0	0	0.07	0	0.11	0.07	0.1	0	0.08	0.03	0.09	0.02	0	0.07	0	0.08	0	0	0	0.07	0.03	0.07	0	0
F42	0	0	0.11	0.15	0	0	0	0.11	0.07	0.05	0	0.06	0.08	0.13	0	0	0	0.08	0	0	0	0.01	0	0.04	0.07	0	0.16	0	0	0	0
F43	0	0	0.02	0	0.04	0.16	0.08	0.07	0.04	0	0.12	0	0.05	0	0.18	0.15	0	0	0	0.24	0	0.07	0	0	0	0	0.14	0.07	0.1	0	0
F44	0.03	0	0.16	0	0	0	0.01	0	0.09	0	0.08	0	0	0	0.21	0.12	0.11	0.05	0.22	0.01	0.11	0	0	0	0	0.01	0	0	0.11	0	0.07
F45	0.05	0	0	0	0	0	0.01	0	0	0.02	0	0.09	0	0	0	0.13	0	0	0.04	0	0.04	0.07	0.04	0	0.01	0.11	0	0	0	0	0
F46	0	0	0.05	0	0	0	0	0	0	0	0	0.07	0	0	0.05	0	0.03	0	0	0.03	0.04	0	0	0	0	0	0.08	0	0	0	0
F47	0.06	0.09	0	0	0	0	0	0.06	0.03	0	0	0	0	0	0.09	0	0	0.06	0	0.07	0	0	0.08	0.15	0.04	0	0	0.1	0	0.08	0.46
F48	0	0	0.01	0	0	0	0	0	0	0.02	0.08	0.15	0	0.21	0.26	0.09	0	0.25	0.07	0	0.12	0.02	0	0	0.11	0	0.07	0.15	0	0.07	0.13
F49	0	0.21	0	0	0.13	0.15	0	0	0.03	0.07	0	0.11	0.07	0	0.17	0	0	0	0.12	0	0	0	0	0.07	0	0	0	0.01	0	0.06	0.05
F50	0	0	0	0	0.14	0.03	0	0.04	0	0.1	0	0	0.03	0	0.02	0	0.01	0.06	0	0	0	0	0.07	0	0.15	0.07	0.06	0	0	0	0.01
F51	0	0.16	0	0.15	0.01	0	0.18	0.1	0.06	0	0	0	0.01	0	0	0.09	0	0.07	0	0.01	0.03	0.01	0.09	0.05	0.01	0	0	0.05	0	0	0
F52	0	0	0	0	0	0.1	0.01	0	0	0	0	0.17	0.05	0	0.03	0	0	0	0.1	0.06	0.1	0.01	0.11	0.09	0.11	0.06	0	0.05	0.09	0.01	0
F53	0	0	0.13	0	0	0.13	0.07	0	0.15	0	0.17	0.18	0	0	0.06	0.05	0.04	0	0	0	0	0.01	0.07	0	0.01	0.08	0	0	0	0.41	0
F54	0.03	0.06	0	0	0	0	0	0	0	0.2	0	0	0.01	0.03	0.04	0	0.03	0	0.01	0.11	0.07	0.12	0.06	0.06	0	0	0.04	0.07	0.25	0.07	0
F55	0	0	0.14	0	0	0.15	0.16	0	0	0	0	0	0	0.02	0	0	0	0	0	0.08	0.1	0.02	0.02	0	0.04	0.18	0.47	0	0	0	0.08
F56	0	0	0.09	0	0.16	0	0	0.02	0	0.3	0.09	0	0.16	0	0	0	0.17	0.06	0	0	0	0.02	0.1	0	0.36	0.04	0	0.01	0	0	0
F57	0	0.22	0.06	0	0.21	0	0	0	0	0.01	0	0	0.07	0	0.04	0.14	0	0	0	0	0.15	0	0.04	0.03	0	0	0	0	0.16	0	0
F58	0	0	0	0	0	0	0	0.04	0.22	0	0	0.08	0.02	0	0	0	0	0	0	0	0.06	0	0	0.25	0.03	0	0	0.06	0.11	0	0.11
F59	0	0	0	0.17	0.04	0.12	0.09	0.04	0	0.07	0	0	0.18	0	0.02	0.01	0	0.06	0	0.04	0	0.02	0.12	0	0	0	0.03	0.07	0	0	0
F60	0.27	0	0	0	0.07	0.03	0	0	0	0	0.07	0.19	0.03	0	0	0.04	0	0	0	0.01	0.01	0.02	0	0	0.14	0.06	0.06	0	0	0.15	0.18
F61	0	0	0.07	0.21	0.04	0	0	0	0	0.02	0	0	0.06	0	0	0	0	0	0.08	0	0	0.03	0	0	0	0.04	0	0	0.03	0	0.03
F62	0.27	0	0.05	0	0	0.07	0	0	0.11	0.08	0	0	0	0.11	0.06	0	0	0	0	0.01	0	0	0.1	0.08	0	0	0	0.06	0.08	0.07	0.19
F63	0	0.23	0.09	0.07	0.07	0	0.3	0.07	0.06	0.1	0.03	0	0.07	0.13	0.04	0	0	0	0	0	0	0	0	0.28	0	0	0	0.06	0.16	0	0.07
F64	0	0	0.13	0.19	0	0	0.27	0.18	0.09	0	0	0	0.01	0.07	0.09	0.09	0	0	0	0.02	0	0.13	0	0.05	0	0.01	0	0	0.07	0	0
F65	0	0	0	0.02	0	0.01	0	0	0.12	0	0	0.01	0.09	0.03	0	0.06	0	0	0	0.09	0	0.01	0	0	0.12	0.08	0	0.01	0	0	0
F66	0.09	0.02	0.07	0.08	0	0	0.04	0	0	0	0	0	0.04	0.01	0	0	0	0	0	0	0	0	0.05	0	0	0.03	0.04	0	0.03	0	0
F67	0.05	0	0	0	0.03	0	0	0	0	0.09	0.11	0	0.01	0.15	0	0	0	0	0.07	0	0.02	0	0.11	0	0	0.27	0	0.02	0.24	0.04	0
F68	0.01	0	0	0	0.23	0	0	0	0.08	0	0.07	0.02	0.09	0	0.04	0.06	0.07	0	0	0	0.11	0.14	0	0.01	0	0	0	0	0	0	0.08
M1	0	0	0.01	0.08	0	0.13	0	0	0.03	0.07	0	0	0.07	0.11	0	0.08	0.06	0.06	0	0	0.06	0.04	0	0	0.05	0.04	0	0	0.01	0	0.23
M2	0	0.29	0	0.01	0.12	0.25	0	0.04	0.05	0.07	0	0	0.28	0	0	0	0	0	0	0.02	0	0.2	0.2	0	0.02	0	0.03	0	0	0.15	0.06
M3	0	0	0	0.04	0	0	0	0	0	0	0	0.13	0.12	0	0	0	0	0	0.27	0	0	0	0.05	0	0	0.1	0	0.02	0.11	0	0
M4	0.06	0	0	0	0	0.06	0.08	0	0	0	0.01	0	0	0	0.05	0.08	0	0.1	0.18	0	0.04	0	0.12	0	0	0	0	0.08	0.03	0	0
M5	0.1	0	0.04	0	0	0	0.07	0.04	0	0	0.14	0.17	0	0	0.22	0.09	0.02	0	0.09	0	0.04	0	0	0	0	0	0	0.05	0	0	0.08
M6	0.07	0.15	0	0.09	0	0.03	0	0.04	0	0	0	0	0.01	0.05	0	0.02	0.02	0	0.08	0.15	0.04	0	0.08	0	0.26	0.16	0	0.15	0	0	0
M7	0.14	0	0	0.02	0.07	0	0	0.11	0	0	0.07	0	0.09	0	0.01	0.01	0	0	0	0	0	0.13	0.03	0	0.07	0	0.15	0	0	0.09	0.13
M8	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0.03	0	0.13	0.09	0	0	0	0	0	0.01	0	0	0.08	0.02	0	0	0
M9	0.22	0.11	0	0	0	0	0.17	0.13	0.16	0	0.02	0.05	0	0	0.17	0	0	0	0.13	0	0	0	0.11	0.19	0.01	0	0.13	0.06	0.08	0	0.23
M10	0	0	0	0	0.07	0	0.06	0	0.12	0.05	0.01	0.15	0.01	0	0	0.04	0	0.03	0.06	0.03	0	0	0	0	0	0.07	0	0.1	0.12	0.06	0
M11	0	0	0.08	0	0.27	0	0.12	0.24	0.14	0.1	0	0	0	0	0.04	0.18	0.22	0	0	0.08	0.02	0.14	0	0	0	0	0.07	0.09	0	0	0.09
M12	0	0.02	0	0.03	0.1	0	0.01	0.04	0	0.1	0	0.09	0.07	0.22	0	0	0	0.02	0	0	0.1	0	0	0	0.02	0.1	0	0	0	0	0.03
M13	0	0	0	0	0.41	0.2	0.02	0.14	0	0	0.01	0.02	0	0	0.2	0	0.05	0	0.03	0.19	0	0.27	0	0.02	0.11	0	0	0	0	0	0.28
M14	0	0	0.1	0.05	0	0.06	0	0.07	0	0.05	0.07	0.02	0	0	0.14	0.03	0.04	0	0.04	0	0	0	0	0.09	0	0.13	0	0.02	0	0.19	0
M15	0	0.12	0.14	0	0	0	0	0	0	0.15	0	0	0.07	0	0	0.07	0.18	0	0	0	0	0.02	0.08	0	0	0	0	0.07	0	0.03	0.06
M16	0.19	0	0	0	0.16	0.07	0	0	0.07	0	0	0	0.03	0	0	0.22	0.12	0.03	0	0	0.18	0	0	0	0	0.06	0.08	0.07	0.07	0	0.05
M17	0	0.14	0.03	0.07	0	0.02	0.14	0	0.02	0.19	0	0.08	0	0	0	0	0.08	0	0.22	0	0.22	0	0.02	0.01	0	0.08	0	0	0	0	0
M18	0	0	0	0	0	0	0.1	0	0	0.07	0	0.02	0	0	0.19	0	0.08	0.08	0	0.27	0	0.06	0	0	0	0	0	0.08	0	0	0
M19	0.06	0	0	0	0.07	0.13	0.46	0.04	0.09	0	0	0.03	0.04	0	0.08	0.1	0	0	0.15	0	0.02	0.01	0.09	0	0.01	0	0.06	0	0.04	0	0
M20	0	0	0	0.07	0	0	0.08	0.04	0	0.15	0.04	0.11	0	0.19	0	0	0	0.16	0	0.1	0.06	0	0	0	0.01	0	0	0.15	0	0	0.05
M21	0.17	0	0.12	0.25	0	0	0.07	0.07	0.15	0	0	0.02	0.11	0	0.01	0	0	0	0	0.03	0.23	0.14	0	0	0	0.07	0.04	0.07	0	0	0.09
M22	0	0.08	0	0	0	0.02	0.03	0.03	0.01	0.18	0.04	0.21	0	0.01	0	0.1	0	0	0	0	0	0	0	0	0.11	0.01	0	0.04	0.08	0	0
M23	0	0.08	0	0.07	0	0	0	0.12	0	0	0.05	0	0	0	0.18	0.13	0.07	0.13	0	0	0	0.16	0	0	0.14	0	0	0	0.06	0.12	0
M24	0.03	0.07	0	0.07	0.25	0.04	0	0	0.04	0.01	0	0	0.08	0	0.04	0.21	0.02	0	0	0.03	0	0.1	0	0.1	0	0.15	0.1	0.07	0	0	0.08
M25	0	0.08	0	0	0	0	0.12	0.06	0.12	0.09	0.03	0	0	0	0.03	0	0.11	0	0	0	0	0	0	0	0.1	0.13	0	0.05	0	0	0
M26	0.11	0	0	0	0.02	0	0.09	0.09	0.1	0.16	0	0	0	0	0.1	0	0.23	0	0.09	0	0	0	0.14	0	0	0	0	0.11	0.2	0.03	0
M27	0	0.16	0.08	0	0.09	0.04	0	0.05	0	0	0	0	0.13	0	0	0.12	0.12	0	0	0	0.19	0	0	0	0	0	0	0	0	0	0
M28	0.09	0	0	0	0	0.02	0.1	0.34	0.18	0	0	0	0	0	0	0.13	0	0	0.14	0	0	0	0.01	0	0	0	0	0.05	0.14	0	0.14
M29	0.01	0	0.02	0	0	0.09	0	0.04	0.07	0.01	0.09	0.08	0	0	0.22	0	0	0	0	0	0	0	0.04	0.09	0.02	0.24	0	0.05	0.08	0.19	0.09
M30	0.33	0.04	0.02	0.05	0.04	0	0.03	0.01	0.07	0	0	0.18	0.02	0.08	0	0	0.04	0	0	0.12	0.02	0	0	0	0.15	0	0	0.08	0.13	0	0.06
M31	0	0	0	0	0	0.06	0.06	0.21	0.08	0	0.06	0	0.01	0	0	0	0	0	0.02	0	0	0	0	0.08	0	0	0	0.01	0.12	0.38	0
M32	0.04	0	0.23	0.02	0	0	0	0	0.1	0.07	0	0.04	0	0.1	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0.09	0.12
M33	0	0	0.04	0	0	0	0.12	0.2	0	0	0	0	0	0.12	0.07	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0	0.14	0
M34	0.12	0.08	0	0	0.15	0	0	0	0.09	0	0	0.09	0.03	0.07	0.13	0.22	0.07	0	0.01	0	0.06	0.07	0	0	0	0	0	0.08	0.03	0	0.02
M35	0	0	0.06	0.01	0	0	0.05	0.17	0	0.14	0.09	0	0.09	0	0	0	0.08	0	0	0.11	0.07	0	0	0	0.1	0	0	0	0	0	0
M36	0	0	0.09	0	0	0	0	0	0	0	0	0	0	0	0.03	0	0	0.07	0	0	0	0	0.01	0	0	0.04	0	0	0	0.17	0.24
M37	0	0.05	0	0.13	0.18	0	0.04	0	0.02	0	0	0.16	0	0	0.29	0	0.13	0	0.13	0	0.17	0	0	0	0	0	0	0	0.12	0	0
M38	0.04	0.21	0.16	0	0.04	0	0	0.05	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0.18	0	0	0.09	0.13	0	0	0
M39	0	0.11	0.05	0	0.07	0	0.04	0.14	0.03	0.09	0	0	0	0	0	0	0.16	0	0	0.11	0.07	0.17	0.05	0.07	0	0	0	0.07	0.03	0.05	0
M40	0	0	0.06	0	0.01	0	0	0.22	0.05	0	0.17	0.09	0	0	0.05	0.03	0	0.04	0	0	0.03	0	0	0	0.19	0.1	0.12	0	0.14	0	0.08
M41	0.1	0	0.04	0	0	0	0	0	0	0.17	0.04	0	0.12	0.11	0	0	0	0	0.02	0.03	0.01	0.28	0	0	0	0	0	0	0	0.18	0
M42	0.01	0.09	0	0.08	0	0	0	0.05	0	0.06	0	0.01	0.04	0.01	0.17	0.11	0.05	0	0.05	0.05	0.18	0.03	0	0.08	0	0.06	0.06	0	0.13	0	0
M43	0	0	0.08	0	0.16	0.04	0	0.07	0	0.16	0.11	0	0	0	0	0	0	0	0	0.13	0.09	0.19	0	0	0.03	0	0	0.04	0	0	0.06
M44	0	0.05	0	0.15	0	0.02	0.11	0.08	0	0.07	0.05	0.05	0.01	0	0.05	0.07	0.03	0.01	0.07	0.06	0	0.11	0.03	0	0.05	0.15	0	0	0.06	0	0
M45	0	0.06	0.07	0	0.14	0	0	0	0	0	0	0.07	0	0	0.15	0	0.02	0.02	0	0	0	0	0	0	0	0	0	0.01	0	0.19	0.07
M46	0.16	0.06	0.13	0.04	0	0	0	0	0.07	0	0	0	0	0	0.05	0.07	0.01	0	0	0	0.05	0.08	0	0.15	0	0	0.01	0.1	0	0	0.1
M47	0	0	0.35	0	0	0.1	0.06	0	0.06	0.05	0	0.06	0	0.04	0	0	0.03	0	0.06	0.1	0.02	0	0	0	0.05	0.12	0	0	0.08	0	0.13
M48	0	0.06	0.05	0	0	0.09	0.07	0.04	0.05	0.08	0.03	0.08	0.07	0.07	0.03	0.11	0.11	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0
M49	0.02	0	0	0.14	0	0	0.04	0	0	0	0	0.06	0	0.37	0.03	0	0.19	0	0	0	0.25	0	0	0.11	0	0.01	0	0.06	0	0.18	0.01
M50	0	0	0.05	0	0	0.09	0.09	0	0.07	0.06	0	0	0.22	0.06	0	0	0	0	0.06	0.15	0	0.02	0.14	0.03	0.1	0.03	0	0.07	0	0.02	0.18
M51	0	0.11	0.1	0	0.02	0.2	0	0	0.13	0.07	0.14	0	0	0	0.02	0	0.28	0.01	0	0.13	0.09	0.04	0	0	0	0	0	0.07	0	0	0
M52	0	0	0	0.03	0	0	0.09	0.02	0.15	0	0.12	0.04	0	0	0.06	0.05	0.07	0.1	0.07	0	0.01	0.11	0	0.03	0	0.24	0	0	0	0	0
M53	0.02	0	0	0.13	0.08	0	0	0	0	0	0	0.05	0	0	0	0	0.03	0.26	0.07	0.09	0.18	0.07	0	0	0.09	0.08	0.03	0	0.07	0	0
M54	0	0	0.05	0	0	0	0	0	0	0.08	0	0	0.07	0.14	0.06	0	0.06	0.03	0.09	0	0	0	0.08	0.01	0.01	0	0.02	0.02	0.07	0.01	0.13
M55	0.03	0	0	0	0	0	0.05	0	0.08	0	0	0.12	0	0	0.07	0	0	0.08	0.03	0	0	0.03	0	0.03	0	0	0	0.01	0.15	0.07	0
M56	0.08	0	0	0	0	0	0.1	0.01	0	0	0.14	0	0	0	0	0	0	0	0.1	0	0	0	0.08	0.07	0.04	0	0	0.05	0.2	0.13	0
M57	0.01	0.08	0.13	0	0	0.16	0	0	0	0	0	0	0	0	0	0	0	0.14	0	0	0.05	0	0.01	0	0	0	0.11	0	0	0	0.04
M58	0.02	0	0.12	0	0.06	0.1	0	0.08	0	0.04	0	0	0	0.11	0	0.15	0	0	0	0.03	0.03	0	0	0.16	0	0	0.02	0	0.04	0.07	0.04
M59	0	0.01	0	0	0.03	0	0.18	0	0.08	0	0	0	0	0	0.01	0.06	0.03	0	0.06	0.09	0	0.05	0	0.15	0.09	0	0	0.01	0	0	0.03
M60	0.04	0.19	0.01	0	0	0	0.14	0	0.05	0.05	0	0.05	0	0.02	0.11	0	0	0	0	0	0	0	0.06	0.18	0	0	0	0.06	0.04	0.05	0.21
M61	0	0.06	0	0.07	0	0.22	0	0	0	0	0.05	0.02	0	0.13	0	0.02	0	0.07	0	0.11	0.04	0.05	0	0	0.05	0.02	0	0	0	0.02	0
M62	0.05	0	0.01	0.08	0.03	0.1	0	0	0.02	0	0	0.06	0.03	0	0	0	0	0	0.12	0.06	0	0.03	0.02	0	0	0	0.03	0.09	0	0	0
M63	0.01	0.02	0.12	0	0	0	0	0	0	0	0	0.15	0	0	0.17	0	0	0.03	0	0	0	0	0.01	0.11	0	0	0	0.03	0	0	0.13
M64	0.03	0	0	0	0	0	0.05	0.23	0.12	0	0.08	0	0	0.22	0	0	0.04	0	0.04	0.06	0	0	0	0	0	0.08	0.04	0	0.23	0.03	0
M65	0	0.02	0.08	0	0	0	0	0.04	0.01	0	0.1	0	0	0	0.04	0	0.02	0	0	0.08	0.05	0	0	0.17	0	0.03	0	0	0.3	0	0
M66	0	0	0	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0.11	0.04	0.03	0	0	0.17	0	0	0.05	0.1	0.06	0	0
M67	0.12	0	0.06	0	0.01	0.06	0	0	0	0.14	0.16	0	0	0.28	0	0	0	0	0	0.01	0.06	0	0.09	0.22	0	0.18	0.18	0	0.22	0	0
M68	0.17	0.54	0.06	0	0.52	0.22	0	0	0.2	0	0	0	0.04	0	0.22	0	0.04	0	0	0.04	0	0.09	0.1	0.18	0.07	0	0.03	0	0	0	0.16
M69	0.07	0	0	0	0	0.01	0	0	0	0	0.01	0.02	0	0	0.08	0.09	0.11	0.02	0	0	0	0.05	0	0	0.17	0.08	0.13	0	0	0	0
M70	0	0	0.13	0	0.01	0	0.09	0.17	0.08	0.08	0.02	0.16	0	0	0	0.09	0	0	0.09	0.01	0.04	0.01	0	0.18	0.02	0	0	0	0.07	0.01	0
M71	0.05	0	0.1	0	0.16	0	0.01	0	0	0	0	0	0	0.03	0	0	0	0	0	0	0.04	0.16	0	0	0	0.04	0.05	0	0	0	0.01
M72	0.02	0	0	0.09	0	0	0	0.06	0.06	0	0.07	0.03	0.02	0	0.08	0.03	0.03	0	0.04	0	0	0.25	0	0	0	0	0	0	0	0	0.03
M73	0.03	0	0	0	0.06	0.08	0	0	0.17	0.04	0	0	0	0.22	0.02	0	0.01	0.2	0	0	0.14	0.24	0	0.1	0	0	0.1	0.12	0.1	0.01	0.07
M74	0.16	0.03	0	0.24	0.11	0	0	0	0.1	0.03	0	0	0.16	0	0	0.11	0	0	0.1	0.23	0.03	0	0	0.02	0	0	0	0.04	0	0	0
M75	0.03	0.09	0	0.07	0	0.19	0.06	0.04	0.09	0	0	0	0	0.11	0	0	0.01	0.13	0	0	0	0.02	0	0.04	0	0	0	0	0	0.15	0.04
M76	0	0.14	0	0	0.06	0.05	0	0	0	0.19	0.08	0	0	0	0	0	0.05	0.07	0	0.14	0.04	0.04	0	0	0.16	0	0.01	0	0.24	0.08	0
M77	0.08	0.13	0.06	0	0.04	0.05	0	0	0	0	0.1	0.03	0.04	0	0	0	0	0	0	0	0	0	0.03	0.07	0	0.01	0.03	0	0.1	0.01	0
M78	0.13	0	0.01	0	0.08	0.15	0	0.03	0	0.12	0.06	0	0.08	0.04	0	0.11	0.03	0	0.11	0	0.02	0.01	0.15	0.06	0.24	0.03	0.06	0	0	0	0.01
M79	0	0	0	0	0	0	0	0.24	0.05	0.06	0	0	0	0	0	0	0.17	0.08	0	0	0.08	0	0.07	0	0.08	0	0	0	0.16	0	0

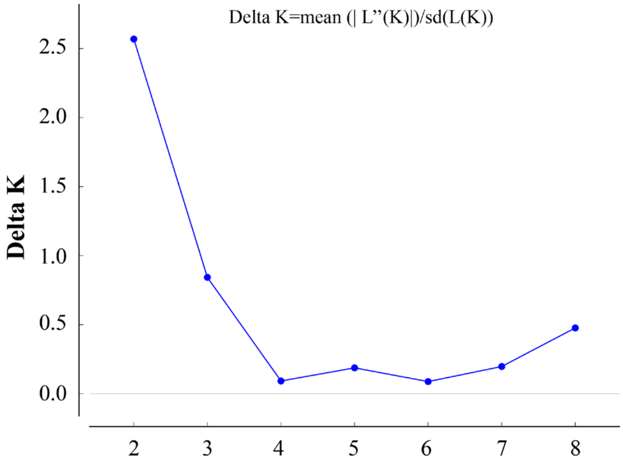

Figure S1 Plots of ΔK as a function of the number of putative groupings (K) in Bayesian population structure analysis of *P. shangchengensis.*